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Kensuke Tsunemitsu*

*Okayama University,

# Tissue typing by unidirectional mixed lymphocyte culture. II. The relationship of in vitro lymphocyte compatibility to skin allograft rejection in dogs*

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**Abstract**

We applied unidirectional MLC test to skin allografts in dogs, and examined the correlation between the strength of the MLC reaction and the survival time of skin allografts. As a result it was found that the skin allografts was rejected within 10 days when the rate of blastformation was more than 18 %. In contrast, the skin graft survived over 13 days when the rate was less than 15 %. The rate of blastformation was inversely correlated with the median survival time of skin allograft.

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# TISSUE TYPING BY UNIDIRECTIONAL MIXED LYMPHOCYTE CULTURE
## II. THE RELATIONSHIP OF *IN VITRO* LYMPHOCYTE COMPATIBILITY TO SKIN ALLOGRAFT REJECTION IN DOGS

Kensuke TSUNEMITSU

*Department of Surgery, Okayama University Medical School, Okayama, Japan (Director: Prof. S. Tanaka)*

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In our previous reports (1) it has been demonstrated that the growth rates of unidirectional mixed lymphocyte culture (MLC) test, using the supersonicated cell homogenate of prospective donor reflects the differences in H-2 antigens. Further, it has been suggested that the cumulative effect of non-H-2 antigens in mice can be verified by this unidirectional MLC test (2).

It may be assumed that the unidirectional MLC test is expedient in estimating the antigen difference as a whole between hybrid animals like dogs as well as humans. Therefore, in the present experiment we applied the MLC test to the skin allograft in dogs, and examined the relationship of growth rate of MLC test to survival time of skin allografts.

## MATERIALS AND METHODS

*Preparation of lymphocytes*: Mongrel dogs of either sex weighing 8—10 kg were used. Prior to the skin graft transplantation approximately 20 ml of whole blood from each dog were drawn under sterile conditions into a syringe containing 0.2 ml of heparin. About 8 ml of plasmagel were added to the whole blood and allowed to sediment for 60 min. at 37°C. The supernatant leucocyte rich plasma was transferred to a test tube and centrifuged at 1500 rpm for 10 minutes. After discarding the supernatant, 5 ml of normal saline solution were added to the leucocyte-rich sediment. The cell suspension was filtered through a glass wool column (or tetron fiber column) to obtain a pure suspension of lymphocytes, and cell count was taken.

This suspension was centrifuged at 1500 rpm for 10 minutes, and an appropriate amount of TC-199 solution (containing 20 % calf serum) was added to the sediment adjusting the cell concentration to $4 \times 10^6$ tells/ml. No effort was made to destroy red cells to obtain a pure suspension of lymphocytes. By the above procedures 82 % of the leucocytes proves to be lymphocytes, and they are proved

100 % live ones by the dye-exclusion test using 0.5 % Tripan blue solution.

*Lymphocyte mixed culture* : The methods employed here were identical with those used in part 1 (2). One ml cell suspension ($4\times10^6$ cells/ml) of prospective recipient was mixed with the suspersonicated cells of prospective donor in the ratio of 1 : 1 (v/v). PHA-M solution was added to culture medium in the concentration of 1 per cent (v/v). The cells were cultured at 37°C for 72 hours, and the percentage of blastformation was assessed morphologically according to the same methods as described in part 1.

*Skin allografts*: In grafting, the skin of a full-thickness 5 cm square was sutured to a prepared bed on the recipient's chest. As a control, an autograft was placed close to the allograft. All animals received a solution (0.5 g) of streptomycin intramuscularly for 7 days after skin grafting. Skin grafts were examined macroscopically every day and biopsy specimens were taken periodically for microscopic examinations. The time when over 50 % healthy part of grafted skin has disappeared is taken as the median survival time, and microscopic cellular infiltrate around the hair follicles and sweat glands as criterion of graft rejection.

## RESULTS

As shown in Table 1, the rate of blastformation ranges from 13.8 % to 22.7 % and the median survival time of skin allografts was from 7 to 14 days. Generally, there was a tendency that the donor skin allograft was rejected within 10 days when the rate of blastformation of recipient cells was more than 18 %, in contrast the skin allograft survived more than 13 days when the rate was less than 15 %. For example, with dog 21 and dog 22 littermate pairs, the rate of blastformation was 21.4 % and the skin allograft survived only 7 days where dog 21 was recipient and dog 22 was donor. On the contrary, the rate was 15.4 % and the survival time was 12 days in the reverse matching of dog 22 as recipient and dog 21 as donor.

Drawing these results in scatter diagram, the rate of blastformation is inversely proportional to the median survival time of skin graft (Fig. 1). The coefficient of correlation is $-0.85$, and is statistically significant, $P<0.05$.

## DISCUSSION

As described in the preface, we have reported that the growth rate of unidirectional MLC test reflects the difference in H-2 antigens of mice (1, 3-5). And further we have shown that the cumulative effect of non-H-2 antigens of mice can be detected by unidirectional MLC test (2).

TABLE 1 THE RELATIONSHIP BETWEEN THE RATE OF BLASTFORMATION AND THE SURVIVAL TIME OF SKIN ALLOGRAFTS IN DOGS

| recipient | donor | survival time of skin allografts | rate of blastformation |
|---|---|---|---|
| 21 | 22 | 7 days | 21.2 % |
| 1 | 2 | 8 | 19.3 |
| 24 | 23 | 8 | 22.7 |
| 6 | 5 | 9 | 19.3 |
| 8 | 7 | 9 | 20.1 |
| 15 | 16 | 9 | 18.9 |
| 23 | 24 | 9 | 16.4 |
| 14 | 13 | 10 | 16.8 |
| 17 | 18 | 10 | 18.3 |
| 20 | 19 | 10 | 18.3 |
| 3 | 4 | 11 | 19.5 |
| 10 | 9 | 11 | 18.8 |
| 12 | 11 | 11 | 18.0 |
| 16 | 15 | 11 | 17.2 |
| 19 | 20 | 11 | 16.6 |
| 2 | 1 | 12 | 16.4 |
| 13 | 14 | 12 | 16.1 |
| 18 | 17 | 12 | 15.8 |
| 22 | 21 | 12 | 15.4 |
| 4 | 3 | 13 | 14.6 |
| 7 | 8 | 13 | 15.1 |
| 9 | 10 | 13 | 13.8 |
| 5 | 6 | 14 | 14.6 |
| 11 | 12 | 14 | 14.1 |
| mean | | 11.1 | 17.2 |

Thus we consider that the unidirectional MLC test is useful in detecting the antigen difference between the donor and the recipient as a whole. In addition to our findings, it has been demonstrated that there is a correlation between the intensity of stimulation in MLC and the rapidity of skin graft rejection in man (6, 7).

On the other hand, it has been reported that the leucocyte matching is significantly correlated with the clinical sequences and the survival of skin or kidney allograft in man (8-10). However, SINGAL *et al.* (11) have demonstrated that the data for parental donors are somewhat less significantly correlated with clinical outcome than in sibling donors. They consider that the lower correlation for the parental donor grafts is not produced by undetected antigens causing rejection, but rather by survival with good function in spite of incompatibilities. As a possible explanation for this,

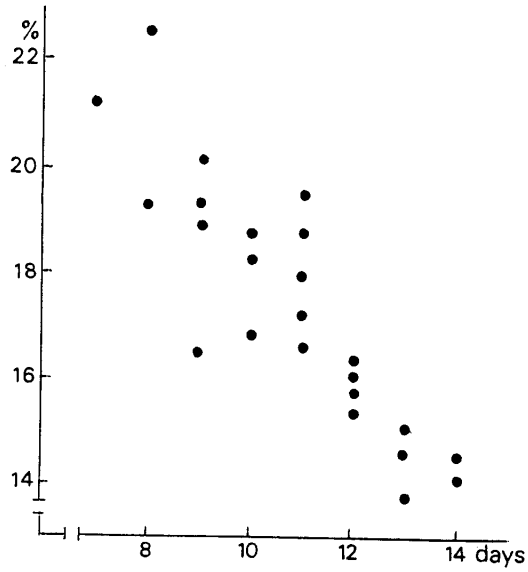


Fig. 1 The relationship between the rate of blastformation and the survival time of skin allografts in dogs

they suggest that HL-A antigens are not "strong" but rather "intermediate" in strength.

Recently, WALFORD *et al.* (12) made a retrospective study of 10 unrelated human pairs showing long and short surviving skin grafts, in which they found no significant correlation between mere numbers of antigenic differences and graft survival. They investigated whether the survival of a graft correlates simply with individual factor differences or perhaps with association of factors forming either complex alleles or subloci of the HL-A system. It is only natural that long versus short survival of the grafts is predictable on the basis of leucocyte typing, when pairs of siblings are selected within the same clone for the exchange skin grafting. It may be reasonable to conclude that leucocyte typing, although still in developmental stage, is useful in the selection of relatively compatible donors, at least for grafts between siblings.

The major histocompatibility locus of dogs still remains obscure, and only a few studies of histocompatibility typing are available (13). RUDOLPH *et al.* (14) employed the MLC method for study of canine histocompatibility antigens, and they produced four cytotoxic antisera to dog lymphocytes by sibling immunization. They substantiated that the results of histocompatibility typing with the MLC method bear a signigicant correlation ($R<0.01$) to those obtained from the typing with four cytotoxic antisera

known to identify important transplantation antigens. They observed also that the presence of additional important histocompatibility antigens in the dog could not be demonstrated serologically with four antisera. RUBINSTEIN *et al.* (15) detected eleven isohemagglutinins induced with canine allografts by Dextran method. They suggest that major transplantation antigens exist in or on the red cells of the dog.

Although we have not been able to detect histocompatibility antigens in dogs, we have observed the genetic disparity between two dogs as a whole by the unidirectional MLC test. The degree of stimulation in MLC test corroborates the rapidity of skin graft rejection. Therefore, we consider that the unidirectional MLC test is of some use in pairing donors and recipients.

## CONCLUSION

We applied unidirectional MLC test to skin allografts in dogs, and examined the correlation between the strength of the MLC reaction and the survival time of skin allografts. As a result it was found that the skin allografts was rejected within 10 days when the rate of blastformation was more than 18 %. In contrast, the skin graft survived over 13 days when the rate was less than 15 %. The rate of blastformation was inversely correlated with the median survival time of skin allograft.

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